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Fate of four Different Classes of Chemicals Under Aerobic and Anaerobic Conditions in Biological Wastewater Treatment

Oladapo Komolafe^{1*}, Wojciech Mrozik², Jan Dolfig³, Kishor Acharya², Lucas Vassalle⁴, Cesar R. Mota⁴ and Russell Davenport²

¹GFL Environmental Inc., Toronto, ON, Canada, ²School of Engineering, Newcastle University, Newcastle Upon Tyne, United Kingdom, ³Department of Mechanical and Construction Engineering, Northumbria University, Newcastle Upon Tyne, United Kingdom, ⁴Departamento de Engenharia Sanitária e Ambiental, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil

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*Correspondence:

Oladapo Komolafe
okomolafe@gflenr.com

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The removal mechanisms and extent of degradation of 28 chemicals (triclosan, fifteen polycyclic aromatic hydrocarbons, four estrogens, and eight polybrominated diphenyl ether congeners) in different biological treatment systems [activated sludge, up-flow anaerobic sludge blanket reactor (UASB) and waste stabilization pond (WSP)] was investigated to provide insights into the limits of engineered biological treatment systems. This was done through degradation experiments with inhibition and abiotic controls in static reactors under aerobic and anaerobic conditions. Estrogens showed higher first order degradation rates (0.1129 h^{-1}) under aerobic conditions with activated sludge inocula followed by low molecular weight (LMW) PAHs (0.0171 h^{-1}), triclosan (0.0072 h^{-1}), middle (MMW) (0.0054 h^{-1}) and high molecular weight PAHs (HMW) (0.0033 h^{-1}). The same trend was observed under aerobic conditions with a facultative inoculum from a WSP, although at a much slower rate. Biodegradation was the major removal mechanism for these chemicals in the activated sludge and WSP WWTPs surveyed. Photodegradation of these chemicals was also observed and varied across the group of chemicals (estrogens (light rate = 0.4296 d^{-1} ; dark = 0.3900 d^{-1}) degraded faster under light conditions while reverse was the case for triclosan (light rate = 0.0566 d^{-1} ; dark = 0.1752 d^{-1}). Additionally, all the chemicals were resistant to anaerobic degradation with UASB sludge, which implies that their removal in the UASB of the surveyed WWTP was most likely via sorption onto solids. Importantly, the first order degradation rate determined in this study was used to estimate predicted effluent concentrations (PECs). The PECs showed good agreement with the measured effluent concentrations from a previous study for these treatment systems.

Keywords: environmental fate, biodegradation, micropollutants, wastewater treatment, effluent quality prediction

INTRODUCTION

Knowledge on the most important mechanisms for micropollutant (chemical contaminants) removal, and the rate of those mechanisms is essential to understand the limits of current wastewater treatment technologies and determine how they might be improved. Biodegradation and sorption have been identified as the main mechanisms of xenobiotic removal in wastewater treatment plants, with volatilization only playing a minor role (Verlicchi et al., 2012). During biological wastewater treatment, micropollutants are either sorbed onto solids or biodegraded—via complete mineralization or incomplete degradation and formation of biotransformation products (Luo et al., 2014). Photodegradation has also been reported as a removal mechanism in waste stabilization ponds (Gomez et al., 2007) and high rate algal ponds (Villar-Navarro et al., 2018). Recently, Falas *et al.* demonstrated that biodegradation was the main fate mechanism for a group of ≥ 20 structurally diverse non-volatile low-sorbing pharmaceuticals in laboratory experiments using unacclimated sludge as an inoculum in different treatment configurations and conditions. However, there has been relatively little systematic research into the fate and mechanisms of chemicals across different use-classes, especially in the context of microbial inocula from range of real full-scale treatment processes (Falås et al., 2016).

In our previous work, the occurrence and removal of 28 chemicals from four different use-classes; steroidal hormones (estrogens- E1, E2, E3 and EE2), personal care products (triclosan), industrial chemicals and flame retardants (polyaromatic hydrocarbons PAHs; and polybrominated diphenyl ethers PBDEs) in different full-scale Brazilian wastewater treatment plants were reported (Komolafe et al., 2021). These plants differed in their treatment processes and energy requirements (from energy-intensive activated sludge systems, to low-energy UASBs plus trickling filters system, and passive energy waste stabilization ponds), and operate under various redox conditions (aerobic, anaerobic and facultative). It is important to get more detailed insight into the fate of the chemicals in these technologically diverse biological treatment systems in order to assess their limits in pursuit of more sustainable and capable removal systems. Such systems would benefit the globe especially low-middle income countries (LMICs) that have little/no wastewater treatment infrastructure but are exposed to an equal or even greater environmental and human health risk from micropollutants.

After initial sorption, biodegradation of estrogen, triclosan, and PAHs removal has been attributed to different mechanisms including deconjugation, co-metabolism with nitrifying biomass or heterotrophic bacteria (Ren et al., 2007; Haritash and Kaushik, 2009; Roh et al., 2009; Racz and Goel, 2010). Furthermore, removal pathways and degradation efficiencies of these chemicals in WWTPs are dependent on the prevailing redox conditions-aerobic, anoxic or anaerobic (Joss et al., 2004; Xue et al., 2010). A review by Liu *et al.* shows that several studies have compared the fate of estrogens under relevant aerobic and

anaerobic wastewater treatment conditions (Liu et al., 2015). However, while the fate of triclosan under aerobic conditions is well documented, there is limited information on its fate under anaerobic conditions especially with anaerobic inocula from real WWTPs (Chen et al., 2011). Most studies on PAH biodegradation have focused on their anaerobic and aerobic digestion in sewage sludge rather than wastewater treatment (Trably et al., 2005; Cea-Barcia et al., 2013). There is also only limited work on the biodegradation of polybrominated diphenyl ethers (PBDEs) under aerobic and anaerobic conditions; Stiborova *et al.* quantified degradation of eight PBDE congeners by activated sludge under aerobic conditions (Stiborova et al., 2015). Furthermore, the fate of these chemicals in WSPs (waste stabilization ponds) is unknown.

The objective of this study was to systematically investigate the biodegradability of the selected group of micropollutants under different redox conditions (aerobic or anaerobic) using biomass from activated sludge, UASB reactors and WSP WWTPs, which are typical of those in warm LMICs. The photodegradation potential of the micropollutants was also investigated with biomass from the WSP. Understanding the extent of degradation (degradation rates) of these chemicals in aerobic and anaerobic systems is required to understand the limits of engineering biological systems (e.g., required hydraulic retention time, HRT) for their removal and predict effluent concentrations (PECs).

EXPERIMENTAL METHODS

Materials

A standard solution of 15 mixed priority PAHs (including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, chrysene, indeno (1,2,3-cd)pyrene, dibenz (a,h)anthracene and benzo (ghi) perylene at 2 mg/ml in dichloromethane), and triclosan (100 mg) were purchased from Sigma, (United Kingdom). A certified standard solution mix of PBDEs (>98% purity) containing eight primary congeners including BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183 and BDE 209 was obtained from Accustandard Inc. via Kinesis (United Kingdom). The concentration of the congeners was 2.5 µg/ml in isooctane, except BDE 209 which was present at 25 µg/ml. Surrogate standards including isotope labeled $^{13}\text{C}_{12}$ -Triclosan (50 µg/ml in methanol), PCB 209 (10 µg/ml in heptane) and 4PC-BDE-208 (50 µg/ml in toluene) (surrogate for PBDEs), and a mix of deuterated PAHs (including acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} and perylene- d_{12} at 2 mg/ml in dichloromethane) were purchased from Wellington Laboratories (via Greyhound Chromatography, United Kingdom), Sigma Aldrich (United Kingdom) and Accustandard (via Kinesis United Kingdom) respectively, with purities higher than 98%. Estrogen standards E1, E2, E3 and EE2 (>98% purity) were purchased from Sigma Aldrich (United Kingdom). Deuterated labeled internal standards of estrogens (E1- d_4 , E2- d_4 , E3- d_2 and

EE2-d₄) were purchased from C/D/N Isotopes (QMX Laboratories, United Kingdom) (>98% purity). Derivatization reagent BSTFA with 1% TCMS was also purchased from Sigma Aldrich (United Kingdom).

Stock solutions were prepared by dissolving the reference and surrogate standards in methanol or acetone. Working solutions were then prepared by diluting the stock solutions in acetone or methanol and dichloromethane or ethylacetate for sample fortification and instrumental analysis respectively (see details of solvent used for different chemicals in the Supporting Information, **Supplementary Information S1**). All solutions were stored at 4°C and allowed to reach room temperature for 15 min before use. Ultra-trace grade of methanol, acetone, MTBE, dichloromethane and isopropanol were obtained from Casa Lab or Biosan (Brazil). Cartridges used for solid phase extraction were Isolute C18 (1000mg, 6 ml) and Isolute C18 (500 mg, 6 ml) were purchased from Biotage (United Kingdom), while Oasis HLB (200 mg, 6 ml) was purchased from Waters (United Kingdom). Glass microfiber filters were purchased from Sartorius (MGB filters, 0.7 mm thick, 1.0 µm particle retention).

Wastewater Treatment Plants and Sampling

Sludge samples were collected from three municipal WWTPs around Belo Horizonte (Brazil) with different treatment processes (activated sludge WWTP, UASB WWTP, and WSP) between December 2016–February 2017. Population equivalent, operational flowrate, plant configuration, hydraulic retention times (HRTs) and solid retention times (SRTs) of these WWTPs are reported elsewhere (Komolafe et al., 2021). A facultative inoculum (inoculum adapted to both aerobic and anaerobic environment) was collected from the facultative pond of the WSP. Samples were collected in cleaned and disinfected (with 1% Virkron for 24 h, followed by several rinses cycles with distilled water), 5 L high-density polyethylene (HDPE) containers; analysis of the empty rinsed containers showed no contamination with any of the target compounds. Samples were stored at 4°C upon arrival at the laboratory and were used within 24 h.

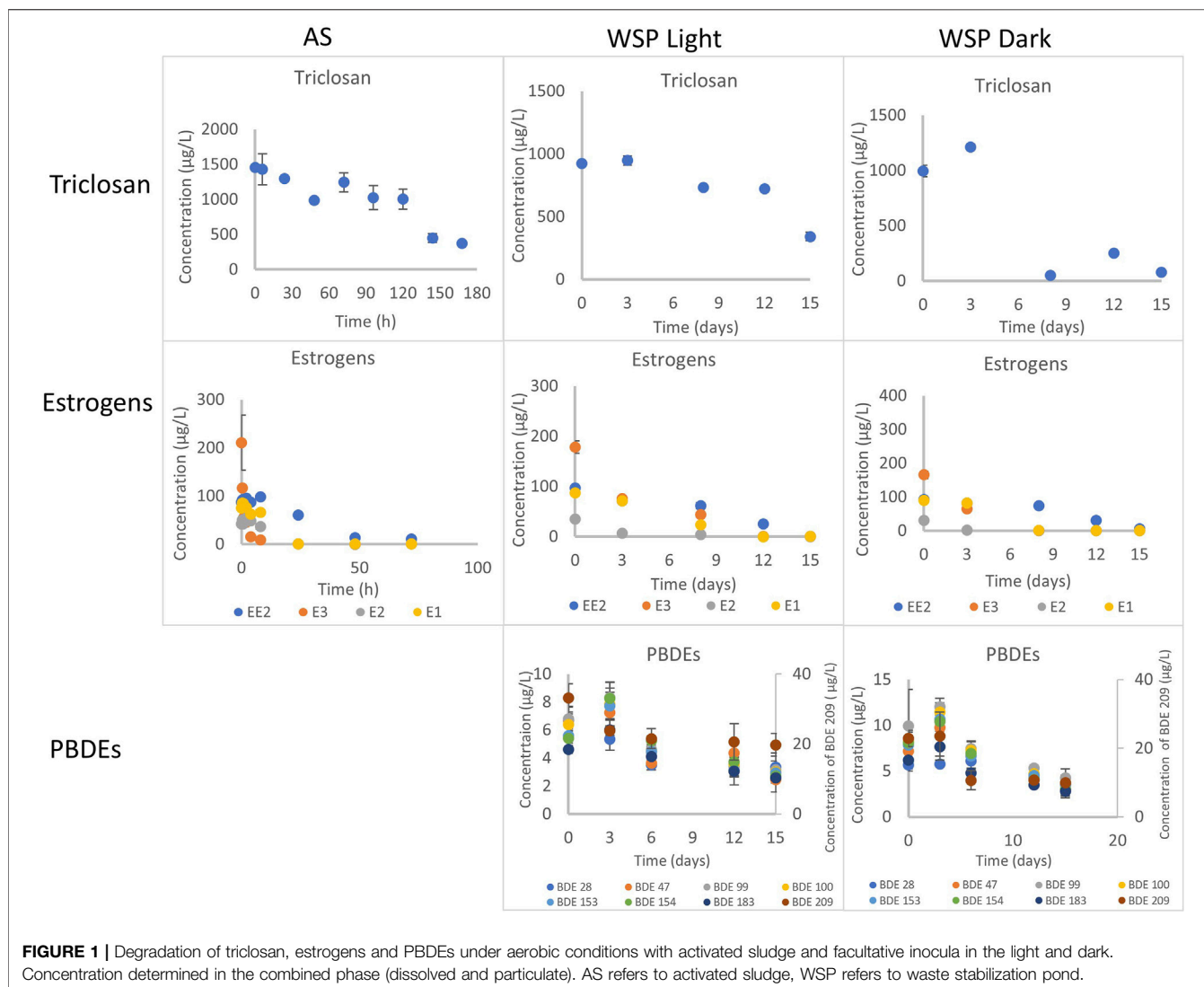
Experimental Design of Biodegradation Assay

Batch reactors (glass test vessels) were used for the degradation experiments as they have previously been used to mimic biotransformation reactions and kinetics in full scale wastewater treatment plants -WWTPs (Helbling et al., 2012). The chemicals were added to test vessels in a solution of methanol or acetone–volume of solvent added was limited to 0.1% to minimize any potential effect. These solvents will not inhibit microbial action as methanol is non-toxic to microorganisms below 1,000 mg/L (Novak et al., 1985; Brasil Bernardelli et al., 2015) and acetone is non-toxic below 5% v/v in different redox conditions (González, 2006). For the aerobic experiment, 1,000 µg/L for triclosan, 200 µg/L for PAHs, 100 µg/L for estrogens and 30 µg/L for

PBDEs were spiked into the test vessels. For the anaerobic experiment, 3,000 µg/L of triclosan, 600 µg/L of PAHs, 300 µg/L for estrogens and PBDEs at 30 µg/L. These concentrations are in the upper range of those found in sludge from municipal WWTPs (Pérez et al., 2001; Bester, 2005; Salgado et al., 2010; Chen et al., 2011; Jones et al., 2014; Pessoa et al., 2014). These high concentrations were chosen to help differentiate between background concentration of the chemicals in the sludge and reduce analytical bias/uncertainties. More details on spiking concentration of the chemicals are provided in the Supporting Information (**Supplementary Information S1**). The experiment was carried out at room temperature (30 ± 2°C) and the temperature for the experiment duration was recorded with a temperature logger (Lascar Electronics, EasyLog EL-USB-2-LCD). For the aerobic biodegradation experiments, dissolved oxygen (DO) and pH were monitored throughout the experiment to ensure that they were not limiting or excessive - especially as pH of the sludge has been reported to affect the adsorption of triclosan (Lindström et al., 2002). The DO and pH for the experiment with activated sludge ranged from 0.25–0.45 mg/L DO and pH of 6.6–7.3 respectively. This WWTP operates at a low dissolved oxygen concentration (0.2–0.3 mg/L) as they are not mandated to remove nitrogen, thereby saving on energy costs - hence the relatively low DO concentration. Furthermore, the total suspended solids (TSS) concentration of the sludge from this plant was 4,290 mg/L. For the experiment with facultative inocula, DO concentration and pH ranged from 6.1 to 7.0 mg/L and 7.5 to 9.5 respectively. The TSS concentration of this inocula was 75 mg/L. Furthermore, inhibition and abiotic controls were employed to check for losses due to non-biological degradation, hydrolysis and volatilization. This was done by autoclaving inocula in test vessels twice (24 h apart) at 121°C and 103 kPa for 20 min as described by Helbling et al. (Helbling et al., 2012). Further details of the aerobic and anaerobic experimental design including test vessels, experimental conditions, equipment and test duration is given in the supporting information (**Supplementary Information S2**).

Extraction and Instrumental Analysis

The compounds were extracted from the wastewater and biosolids by SPE and were analyzed by gas chromatography with mass spectrophotometry (GC-MS- for triclosan and PAHs), liquid chromatography with mass spectrophotometry (LC-MS/MS- for estrogens) or gas chromatography with electron capture detector (GC-ECD-for PBDEs) (Komolafe et al., 2019; Komolafe et al., 2021). The sludge was unfiltered before extraction to account for the chemicals in both the dissolved and solids/particulate phase. Information on the extraction and instrumental analysis of triclosan, PAHs, estrogens and PBDEs is reported elsewhere (Komolafe et al., 2021). SPE cartridges were stored at –20°C in Brazil, then transported in boxes with cooling packs to the UK, where elution and instrumental analysis was performed.



Quality Assurance and Data Analysis

Method accuracy was evaluated by performing recovery experiments in blanks (deionized water, $n = 3$) and matrix samples (final effluent and activated sludge, $n = 3$) at different fortification levels (**Supplementary Information S1**). The repeatability of the method was determined by the relative standard deviation (% RSD) from the recovery experiments in the fortified blank and matrix sample. The recovery rate of triclosan was 102% in effluent (RSD = 11%), and 101% in activated sludge (RSD = 2.5%) (**Supplementary Tables S1, S3**). The recoveries of PAHs ranged from 67 to 107% (RSD = 0.8–11%) in effluent, and 67–101% (RSD = 0.8–8.1%) in activated sludge (**Supplementary Tables S2, S3**). Recoveries of PBDEs and estrogens are reported elsewhere (Coello-Garcia et al., 2019; Komolafe et al., 2019). Method IDL, MDL and MQL have previously been reported (Komolafe et al., 2021). All samples were fortified with labeled internal standards- $^{13}\text{C}_{12}$ - triclosan, $^{13}\text{C}_{12}$ - methyl triclosan, acenaphthene- d_{10} , phenanthrene- d_{10} ,

chrysene- d_{12} , E1- d_4 , E2- d_4 , E3- d_2 , EE2- d_4 and perylene- d_{12} (except for PBDE analysis where unlabelled standards PCB 209 and 4PC-BDE-208 was used) for possible correction of matrix effect and losses during sample extraction. Five levels of concentration for each analyte was used for the calibration curve, and linearity was observed when the correlation coefficient was >0.99 .

The reaction kinetics was evaluated by checking the fit of the degradation experimental data to zero-order, first-order, second-order and third-order. The degradation kinetics for all the chemicals follow first-order kinetics with the best linear regression values (R^2) (See **Supplementary Information S2, Table S1**).

The obtained first-order biodegradation rates were used to predict effluent concentrations and assess the likelihood of risk when these chemicals are discharged into water bodies. In a completely stirred tank reactor (CSTR), effluent concentration is given by the equation below (Levenspiel, 1999);

TABLE 1 | Degradation rates of different classes of chemicals under aerobic conditions with activated sludge inocula.

Compound	Chemical class ^a	First order rates (h ⁻¹)	t _{1/2} (h)	Class average rate (h ⁻¹)
Triclosan	PCP	0.0072	96.3	0.0072
Naphthalene	LMW PAHs	0.0340	20.4	0.0171
Acenaphthylene	LMW PAHs	0.0180	38.5	
Acenaphthene	LMW PAHs	0.0165	42.0	
Fluorene	LMW PAHs	0.0134	51.7	
Phenanthrene	LMW PAHs	0.0126	55.0	
Anthracene	LMW PAHs	0.0082	84.5	
Fluoranthene	MMW PAHs	0.0063	108.3	0.0054
Pyrene	MMW PAHs	0.0038	177.7	
Benz(a)anthracene	MMW PAHs	0.0050	135.9	
Chrysene	MMW PAHs	0.0060	113.6	
Benzo(b)fluoranthene	HMW PAHs	0.0053	126.0	0.0033
Benzo(a)pyrene	HMW PAHs	0.0032	203.8	
Indeno (1,2,3-cd)pyrene	HMW PAHs	0.0028	216.6	
Dibenz (a,h)anthracene	HMW PAHs	0.0020	315.0	
Benzo (g,h,i)perylene	HMW PAHs	0.0023	288.8	
EE2	Steroidal hormones	0.0331	20.9	0.1129
E1	Steroidal hormones	0.1496	5.5	
E2	Steroidal hormones	0.1426	4.6	
E3	Steroidal hormones	0.1261	4.9	

^aPCP represents personal care product; LMW, MMW and HMW represents low molecular weight, middle molecular weight and high molecular weight respectively, and are industrial chemicals.

$$\text{Predicted effluent concentration} = \frac{\text{Influent concentration}}{1 + k \times \text{HRT}}$$

Where k = biodegradation constant (h⁻¹), HRT = hydraulic retention time of the activated sludge plant sampled, and concentrations are in ng/L.

RESULTS AND DISCUSSION

Biodegradation Under Aerobic Conditions Triclosan

Biotransformation of Triclosan With Activated Sludge Inocula

Under aerobic conditions, triclosan concentration in the batch tests decreased by 74% over the duration of the experiment (168 h) (Figure 1, Supplementary Figure S1). Chen *et al.*, reported a similar observation of 86% degradation of triclosan spiked at 500 µg/L after 168 h (Chen *et al.*, 2011). Interestingly, this low DO (0.2–0.45 mg/L) activated sludge inocula performed like a traditional activated sludge inocula with DO of 4 mg/L and above (Bester, 2005; Chen *et al.*, 2011). This implies that activated sludge-based treatment plants operating with low DO concentrations (typical in warmer climates with no nitrogen removal requirements) can remove triclosan as effectively as the traditional ones. The biodegradation of triclosan followed first order kinetics with a rate constant of 0.0072 h⁻¹ and an estimated half-life of 96 h (Table 1, Supplementary Table S5). This rate is similar to those reported by (Chen *et al.*, 2011), but slower than those reported by (Armstrong *et al.*, 2018)—perhaps due to the two fold lower starting concentration (Supplementary Table S5).

Methyl triclosan was produced in the batch tests, and concomitantly increased with decreasing triclosan concentration (Supplementary Figure S1), thereby supporting reports that methyl triclosan is biotransformation product of triclosan under aerobic conditions (Heidler and Halden, 2007). Methyl triclosan is known to be more persistent, bio-accumulative and lipophilic than triclosan (Lindström *et al.*, 2002; Balmer *et al.*, 2004). However, only 4.8% of triclosan was bio-transformed to methyl triclosan, which suggests the formation of other major triclosan transformation products. Phenol, catechol and 2, 4- dichlorophenol have previously been identified as the major bio-transformation products of triclosan under aerobic conditions with pure bacteria strains isolated from activated sludge (Veetil *et al.*, 2012). The observed methylation of triclosan in this study (4.8%) was higher than the 1% reported by (Chen *et al.*, 2011) but lower than the 42% reported by (Armstrong *et al.*, 2018).

Surprisingly, results from the inactivated control (autoclaved sludge) showed losses after 168 h. This was possibly due to incomplete inactivation of the inocula during the adopted autoclaving process (Helbling *et al.*, 2012). The methylation of triclosan observed in the inhibition control (Supplementary Figure S1) indicated incomplete inactivation of the sludge, since such a transformation is unlikely to occur without a biological catalyst. Therefore, associated reductions of triclosan in this control may be attributed to biodegradation, not abiotic losses. Experimental data from previous UK studies (where controls worked) also indicated that removal of triclosan was by degradation (data not shown). Hence, reduction observed was mainly due to degradation. Furthermore, sorption contributes majorly to triclosan removal in wastewater as our previous study

TABLE 2 | Degradation rates of different classes of chemicals under aerobic conditions with facultative pond inocula.

Compound	Chemical class	First order rates (d ⁻¹)	Class average rate (d ⁻¹)	First order rates (d ⁻¹)	Class average rate (d ⁻¹)
		Light		Dark	
Triclosan	PCP	0.0566	0.0566	0.1752	0.1752
Naphthalene	LMW PAHs	0.289	0.2707	0.3046	0.2645
Acenaphthylene	LMW PAHs	0.3129		0.3337	
Acenaphthene	LMW PAHs	0.3443		0.3833	
Fluorene	LMW PAHs	0.2472		0.2641	
Phenanthrene	LMW PAHs	0.2995		0.2111	
Anthracene	LMW PAHs	0.1313		0.0904	
Fluoranthene	MMW PAHs	0.0863	0.0576	0.1024	0.0620
Pyrene	MMW PAHs	0.0787		0.0947	
Benz(a)anthracene	MMW PAHs	0.0347		0.0257	
Chrysene	MMW PAHs	0.0305		0.0253	
Benzo(b)fluoranthene	HMW PAHs	0.033	0.0364	0.0173	0.0200
Benzo(a)pyrene	HMW PAHs	0.049		0.0385	
Indeno (1,2,3-cd)pyrene	HMW PAHs	0.0388		0.0169	
Dibenz (a,h)anthracene	HMW PAHs	0.0322		0.009	
Benzo (g,h,i)perylene	HMW PAHs	0.0291		0.0184	
EE2	Steroidal hormones	0.3024	0.4296	0.1632	0.3900
E1	Steroidal hormones	0.4368		0.3888	
E2	Steroidal hormones	0.4872		0.5016	
E3	Steroidal hormones	0.4920		0.5064	
BDE 28	BFR	0.0666	0.0551	0.0599	0.0644
BDE 47	BFR	0.0612		0.0692	
BDE 99	BFR	0.0626		0.0648	
BDE 100	BFR	0.059		0.0677	
BDE 153	BFR	0.0575		0.0645	
BDE 154	BFR	0.0578		0.0670	
BDE 183	BFR	0.0479		0.0616	
BDE 209	BFR	0.0285		0.0604	

BFR, brominated flame retardants; LMW, low molecular weight; MMW, middle molecular weight; HMW, high molecular weight; PCP, personal care product and are industrial chemicals.

reported that 59–92% of triclosan was sorbed onto suspended solids (Komolafe et al., 2021).

Biotransformation of Triclosan With WSP (Facultative) Inocula

The concentration of triclosan decreased by 63 and 92% under light and dark conditions respectively in 15 days with inocula obtained from the WSP (WWTP C) (**Figure 1**).

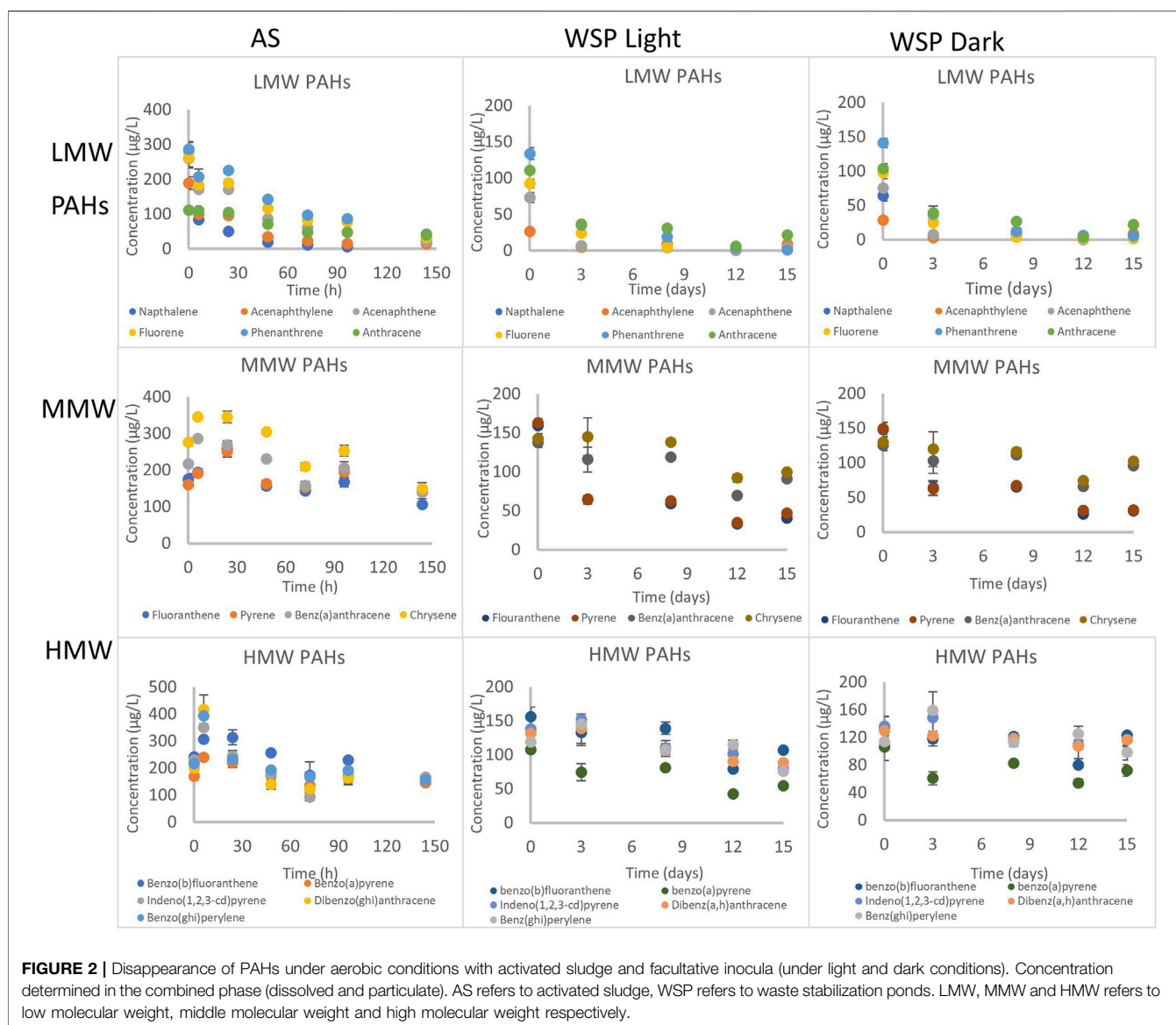
The biodegradation of triclosan followed first order kinetics under both conditions with half-life of 12 days (rate constant of 0.0566 d⁻¹) in the light and 4 days (rate constant of 0.1752 d⁻¹) in the dark (**Table 2, Supplementary Table S6**). Photodegradation has been reported to play a role in triclosan elimination from the environment due to its degradation when irradiated with UV light and sunlight (Chen et al., 2008; Buth et al., 2010; Tamura and Yamamoto, 2012). A study reported 63% photodegradation of triclosan (at a first order rate of 0.087 d⁻¹) and formation of 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) in fresh water after irradiation with white light (fluorescent lamp), and no degradation under dark conditions (Aranami and Readman, 2007). In contrast, triclosan degraded faster under dark conditions in this study using a facultative inoculum. This might be due to more intense competition between autotrophs and heterotrophs for nutrients under light conditions leading to less degradation of triclosan. On the other hand, the death of

autotrophs and subsequent release of nutrients might have favored the co-metabolic degradation of triclosan by the heterotrophs. Anaerobic conditions were not maintained in these experiments and being an inoculum sourced from a facultative pond, the co-existence of aerobic and anaerobic conditions might have confounding effects on the system. Similar to the aerobic experiment with activated sludge, the inactivated control (autoclaved inocula) also showed reduction of triclosan over time (data not shown), most likely for the same reasons as in the activated sludge aerobic experiment above. Hence, reductions observed was mainly due to degradation.

Polycyclic Aromatic Hydrocarbons

Biotransformation of Polycyclic Aromatic Hydrocarbons With Activated Sludge Inocula

Reduction of low molecular weight (LMW) PAHs ranged from 64% for anthracene to 97% for naphthalene after 144 h (**Figure 1, Supplementary Figure S3**). By comparison, reduction of middle molecular weight (MMW) PAHs ranged from 57% for chrysene to 61% for fluoranthene (**Figure 2, Supplementary Figure S4**), while high molecular weight (HMW) PAHs ranged from 56% for benzo(b)fluoranthene to 21% for dibenz (a,h)anthracene (**Figure 2, Supplementary Figure S5**). The inactivated control (autoclaved activated sludge) showed reduction of some LMW



PAHs (naphthalene, acenaphthylene, acenaphthene and fluorene) - suggesting that volatilization contributed to the reduction of LMW PAHs. Volatilization tendency for these four chemicals was estimated to be between 0.5–2% at 25°C and atmospheric pressure (see calculation in S4), but aeration during activated sludge treatment has been reported to intensify volatilization rates (Luo et al., 2014). Lighter PAHs tend to volatilize as a result of their relatively lower melting point and higher water solubility compared to heavier PAHs (Trably et al., 2005). The inactivated control for MMW and HMW PAHs showed no losses (Supplementary Figures S3, S4) indicating that reductions observed were solely due to degradation. However, sorption contributes majorly to the removal of PAHs in wastewater as our previous study reported that 60–97% of PAHs was sorbed onto suspended solids (Komolafe et al., 2021).

The biodegradation of PAHs followed first order kinetics and their half-lives ranged from 20 to 315 h (Table 1, Supplementary

Table S7). The half-lives and first order rate constants of LMW PAHs ranged from 20 to 85 h (naphthalene to anthracene) and 0.0340–0.0082 h⁻¹ (naphthalene to anthracene) respectively. In comparison, slower rates and longer half-lives were observed for MMW PAHs with half-lives and rate constants ranging from 110 to 182 h (fluoranthene-pyrene) and 0.0063–0.0038 h⁻¹ (fluoranthene-pyrene) respectively (Table 1). HMW PAHs were the slowest to degrade with half-lives and rate constants ranging from 131 to 347 h and 0.0053–0.0020 h⁻¹. This further suggests that degradation rates of PAHs increases from light to heavier PAH under aerobic conditions, and agrees with findings by several other reports (Trably et al., 2005; Ghosal et al., 2016).

Biotransformation of Polycyclic aromatic hydrocarbons With WSP (Facultative) Inocula

Degradation of 15 PAHs under aerobic conditions with facultative inocula was observed in the presence and absence of white light

(fluorescent bulb placed 20 cm from the reactors) (Figure 2). The inactivated control (autoclaved sludge) failed in this experiment. However, some experimental data from previous UK studies (data not shown) showed that removal was mostly by degradation with minor volatilization. The reduction of LMW PAHs ranged from 80 to 98% and 79–99% (anthracene to naphthalene) under light and dark conditions respectively (Figure 2, Supplementary Figure S6). In comparison, the reduction of MMW PAHs ranged from 30 to 74% and 22 to 79% (chrysene to fluoranthene) under light and dark conditions respectively (Figure 2, Supplementary Figure S7). The extent of degradation of HMW PAHs was lowest, ranging from 31 to 45 and 8.2 to 32% (benzo(b)fluoranthene to benzo(a)pyrene) under the light and dark conditions respectively (Figure 2, Supplementary Figure S8).

The degradation of PAHs followed first order kinetics with half-lives ranging from 2 to 34 days and 2 to 77 days under light and dark conditions (Table 2, Supplementary Table S8). Degradation was slower with increasing molecular weight of the PAHs since rate constants were between 0.0863–0.3833 d⁻¹, 0.0253–0.1024 d⁻¹ and 0.0090–0.0490 d⁻¹ for LMW, MMW and HMW PAHs respectively (Table 2). Similar degradation rates and half-lives were observed for LWM PAHs under light (2 days) and dark (2 days) conditions with similar rate constants, except for phenanthrene and anthracene, where the reaction was relatively faster in the light (half-life = 2 and 5 days respectively in light, 3 and 8 days in the dark) (Table 2). The degradation of MMW PAHs was faster under light (half-lives ranging from 8 to 23 days) than dark conditions (half-lives ranging from 7.7–27 days). Similarly, degradation of HMW PAHs was faster in the light (half-lives between 14–24 days), than dark conditions (half-lives between 18–77 days).

Photolysis has been reported as a major abiotic degradation process for PAHs either via direct photooxidation (radiation absorbed directly by PAHs) or photosensitization (transformation mediated by other light absorbing substances) (Bertilsson and Widenfalk, 2002; Saeed et al., 2011). This explains the observed faster degradation under light conditions. Furthermore, photolytic degradation has been reported to be more rapid in higher molecular weight PAHs due to their lower quantum yields that leads to increased photoreactivity by a better overlap of their adsorption to solar spectrum (Kochany and Maguire, 1994). This also explains the extent of high molecular weight PAHs photodegradation in presence of light compared to low and middle molecular weight PAHs in this study.

Estrogens

Biotransformation of Estrogens With Activated Sludge Inocula

The reduction of all four estrogens observed in the experiment was solely due to biodegradation as no sign of abiotic losses was indicated by the inactivated control (autoclaved sludge) (Figure 1, Supplementary Figure S10). The concentration of E1, E2, E3 decreased by 99% in 72 h in the batch tests; with the most reduction (96–99%) occurring within 24 h (Supplementary Figure S10). EE2 was also reduced by 88% after 72 h

(Supplementary Figure S10). Rapid transformation and high removal rate of E3, E2, E1 and EE2 has been reported to occur under nitrifying conditions with activated sludge and ammonia oxidizing bacteria (Dytczak et al., 2008; Gaulke et al., 2008; Haiyan et al., 2007). The simultaneous oxidation/removal of NH₄⁺-N and organic micropollutants is a consequence of co-metabolic biotransformation induced by autotrophic aerobic bacteria present in the system (Fernandez-Fontaina et al., 2012; Helbling et al., 2012; Yi et al., 2006). Some authors suggested that the enzyme ammonium monooxygenase (AMO) is the catalyst responsible for the micropollutants and NH₄⁺-N co-metabolism (Yi et al., 2006). In our study (Supplementary Figure S10), E3 and E1 were the least adsorbed estrogen as 96–97% and 92–96% respectively was present in the aqueous phase, compared to EE2 (25–47%) and E2 (8.2–8.5%). This might be due to their water solubility and Log K_{ow} (2.47 for E3, 3.13 for E1, 3.67 for EE2 and 4.10 for E2) (Liu et al., 2009) and implies that the relatively highly hydrophobic EE2 and E2 possess higher tendency of adsorption when compared to E3 and E1, hence, their removal in treatment plants can be partly due to adsorption (Urase and Kikuta, 2005; Wang et al., 2013).

The biotransformation of the all four estrogens followed first order kinetics with estimated half-lives of 5.5, 4.6, 4.9, and 20.9 h for E1, E2, E3 and EE2 respectively (Table 1, Supplementary Table S5). Reported degradation rates of estrogens varies greatly in the literature because the biodegradation assays were conducted under different temperatures, inocula concentration and spiked concentrations (Liu et al., 2015) (Supplementary Table S5). The degradability of the estrogens in our study is E3/E2 > E1 > EE2. This observation is in agreement with previous studies (Petrie et al., 2014; Liu et al., 2015). However, Shi et al., reported that E3 was the most resistant to aerobic degradation (Shi et al., 2004) (Supplementary Table S9).

Biotransformation of Estrogens With WSP (Facultative) Inocula

The reduction of all four estrogens was observed in the aerobic batch tests under light and dark conditions (Figure 1, Supplementary Figure S11). Although, no other studies have used facultative inocula in their experiments, the degradation trend observed was similar to those reported with activated sludge inocula (Dytczak et al., 2008). About 99.5% of E1, E2 and E3 were degraded after 15 days under the light and dark conditions (Supplementary Figure S11). Also, more EE2 was degraded under light conditions (99.5%), than in the dark (93.5%) after 15 days (Supplementary Figure S11). Most of the losses of E1, E2 and E3 (>95%) occurred within 8 and 12 days under dark and light conditions respectively. However, degradation of EE2 was slower, with 93.5–99.5% removal after 15 days.

The degradation of all four estrogens under light and dark incubation conditions followed first order kinetics (Table 2, Supplementary Table S10). The reaction kinetics was similar for E1 and E3 under both light and dark conditions with estimated rate half-lives of 1.4 days (Table 2). Meanwhile the degradation reaction kinetics was faster under light conditions for E2 and EE2. The rate constant of E2 (half-life of 1.6 days) was 0.4368 d⁻¹ under light conditions, compared to 0.3888 d⁻¹

(half-life of 1.8 days) under dark conditions. Also, the degradation rate almost doubled in the light ($k = 0.3024 \text{ d}^{-1}$, half-life = 2.3 days) for EE2 compared to the dark conditions ($k = 0.1632 \text{ d}^{-1}$, half-life = 4.2 days) (Table 2).

This enhanced degradation E2 and EE2 under light conditions was due to photolysis in which the compounds might have degraded directly by absorption of photons or through photosensitization (Zhang and Zhou, 2005; Sornalingam et al., 2016). A review of several publications on the photodegradation of estrogens published recently by Sornalingam *et al.* reports photodegradation of estrogens under different light sources (sunlight, visible light, UV light-with UV light most effective) and water matrixes (Sornalingam et al., 2016). It was established that the rate of photodegradation of estrogens is influenced by light source and intensity, and solution matrix (Sornalingam et al., 2016). In fact, the same light source was reported to have different effects on individual estrogens, as E1, E2 and EE2 were removed at a similar rate under catalysis, but removal followed the order $\text{EE2} > \text{E1} > \text{E2}$ under UVA light (Coleman et al., 2004). This might explain the observed photodegradation of E2 and EE2, and not for E1 and E3 as individual chemicals can behave differently.

Polybrominated Diphenyls

Due to a limited inventory of PBDE analytical standard and unavailability in Brazilian markets at the time of the study, the degradation experiment for PBDEs was only carried out with WSP facultative inocula. Experiment with activated sludge inocula was not performed.

Biotransformation of Polybrominated Diphenyls With WSP (Facultative) Inocula

Reduction in the concentration of individual PBDE congeners ranged from 40 to 63% and 42–63% under light and dark conditions respectively (Figure 1, Supplementary Figure S13). Furthermore, degradation of the most abundant congener BDE 209 was 40 and 57% under light and dark conditions respectively. Similar results were reported by Stiborova *et al.* where degradation of BDE 28 to BDE 209 was between 62–78% after 11 months of studies in aerobic reactors inoculated with sewage sludge and placed in the dark (Stiborova et al., 2015). The abiotic control showed no losses, hence, reduction observed for the congeners was due to degradation. However, sorption contributes majorly to the removal of PBDEs in wastewater as our previous studies reported that 20–87% of PBDEs were sorbed onto suspended solids (Komolafe et al., 2019; Komolafe et al., 2021).

The degradation of the PBDE congeners followed first order kinetics with half-lives ranging from 10.4 to 24.3 days and 10–11.5 days under light and dark conditions respectively (Table 2, Supplementary Table S7). The degradation rates were relatively faster in the dark conditions with rate constants between $0.0599\text{--}0.0692 \text{ d}^{-1}$ and $0.0285\text{--}0.0666 \text{ d}^{-1}$ in dark and light conditions respectively. This was true for all the congeners except BDE 28, where the rate was faster under white light. This is surprising as photodegradation has been suggested as an important abiotic transformation process for

PBDEs in the environment, whereby heavily brominated congeners (such as BDE 209, BDE 183) undergo debromination to less brominated ones (such as BDE 28, BDE 47) (Ahn et al., 2006; Davis and Stapleton, 2009; Pan et al., 2016).

A recent review on photodegradation of PBDEs by Pan *et al.* showed reductive debromination of PBDEs in different aquatic systems with a range of irradiation sources (xenon or mercury or UV lamps, sunlight (Pan et al., 2016). They also suggested that chemical species such as humic substances and ions (halides and metals) present in the aquatic systems strongly influences photochemical transformations. Furthermore, the source and intensity of light affects photolytic degradation of pollutants (Sornalingam et al., 2016). In this study, the chemical species of the system and the light source (white fluorescent light) might not have facilitated photodegradation, and the observed losses of PBDEs was biodegradation by the microbial community. Furthermore, the increased reaction kinetics in the dark might have been due to the death of autotrophs over time leading to increased availability of nutrients for the heterotrophs to co-metabolically degrade the PBDEs.

Comparing the Degradation Rates of the Different Classes of Chemicals Under Aerobic Condition

With activated sludge inocula, estrogens degraded more rapidly with their average reaction rate 7, 16, 21 and 34 times higher than those for low molecular weight (LMW) PAHs, triclosan, middle molecular weight (MMW) PAHs and high molecular weight (HMW) PAHs respectively (Table 1, Figure 3). LMW PAHs degraded two times faster than triclosan on average. Degradation of triclosan was slightly faster (1.3 times) than MMW PAHs but was 2 times faster than HMW PAHs (Table 1, Figure 3). The difference in chemical structure and functional groups among the chemical classes influenced their biodegradation abilities. Under aerobic conditions, biotransformation of aromatics is initiated by hydroxylation (addition of molecular oxygen to the aromatic ring) followed by cleavage of the aromatic ring and then electrophilic substitution (Pitter and Chudoba, 1990). The presence of strong electron withdrawing substituent groups (such as halogens, nitrogen) decreases the electron density of the aromatic ring and consequently reduces the biodegradation rate in comparison to weaker substituent groups (OH, CH_3) (Pitter and Chudoba, 1990). This explains why the degradation of triclosan was slower than estrogens and some PAHs. Vuono *et al.*, reported poor removal of chemicals with strong electron withdrawing functional groups in full scale membrane bioreactors in comparison to other chemicals (Vuono et al., 2016).

A similar trend was observed with facultative pond inocula where the degradation of estrogens was 2–3, 6–8, 12–20 and 2–8 times faster than LMW PAHs, MMW PAHs, HMW PAHs and triclosan respectively under light and dark incubation conditions (Table 2). Furthermore, the degradation of PAHs was 3–4 times faster than PBDEs under both incubation conditions. In summary, the degradation rate of the chemicals follows this order-estrogens > PAHs > triclosan > PBDEs. The

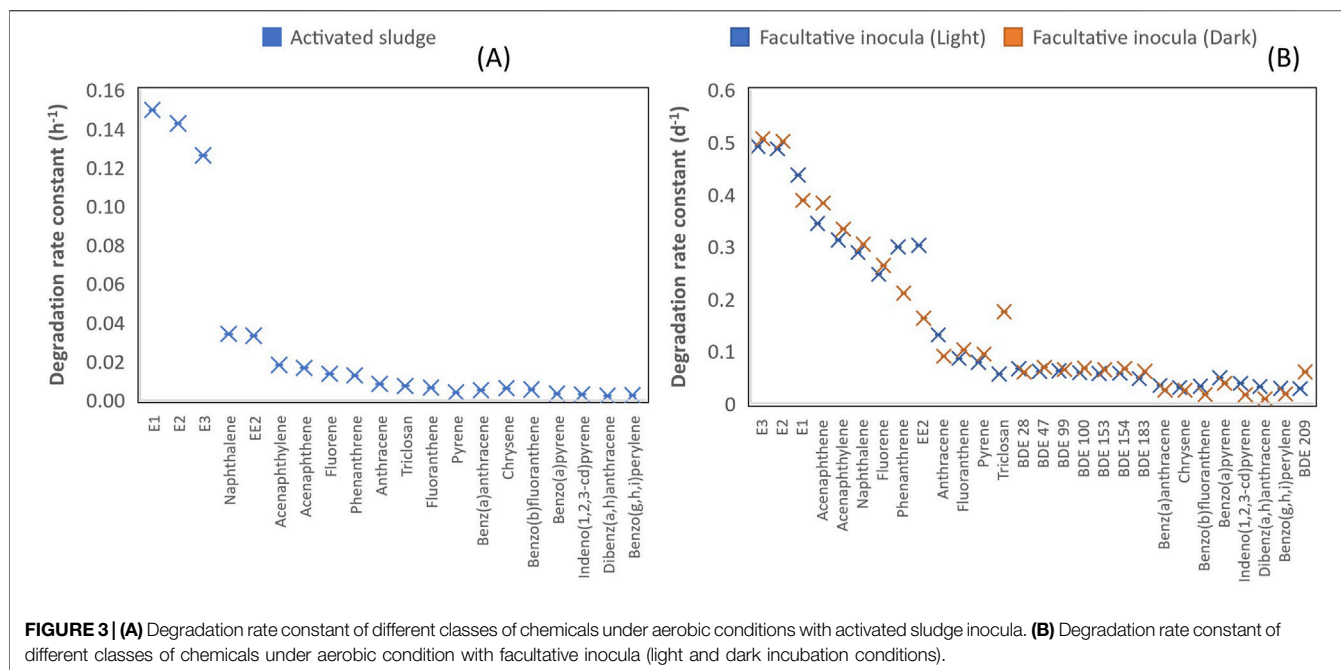


FIGURE 3 | (A) Degradation rate constant of different classes of chemicals under aerobic conditions with activated sludge inocula. **(B)** Degradation rate constant of different classes of chemicals under aerobic condition with facultative inocula (light and dark incubation conditions).

pseudo first-order rates of all the classes of chemicals under aerobic conditions with activated sludge and facultative inocula was also estimated with the suspended solids concentration of the inocula. The pseudo-first order rate ranged from 0.0005–0.0349 L.gSS/h for activated sludge inocula, 0.3800–6.560 L.gSS/d for facultative inocula under light conditions and 0.1200–0.675 L.gSS/d facultative inocula under dark conditions (Supporting Information, **Supplementary Table S9**).

Biodegradation Under Anaerobic Conditions

After 15 days of incubation, no reduction was observed in the concentration of triclosan (**Supplementary Figure S2**), PAHs (**Supplementary Figure S9**), estrogens (**Supplementary Figure S12**) and PBDEs (**Supplementary Figure S14**). Also, methylation of triclosan was not observed in the anaerobic batch tests (results not shown). Chen *et al.* also reported no significant biodegradation of triclosan under anaerobic conditions with mixed culture inocula (Chen *et al.*, 2011). However, 60–90% degradation of triclosan under anaerobic conditions by pure bacteria strains inoculated from anaerobic sludge has been reported (Veetil *et al.*, 2012). Some studies have reported anaerobic degradation of estrogens, especially E1 and E2 (Andersen *et al.*, 2004; Zhang *et al.*, 2015), while other studies reported the resistance of EE2 to anaerobic degradation (Joss *et al.*, 2004; Czajka and Londry, 2006). Furthermore, there has been no previous report on anaerobic degradation of E3. Either activated sludge or lake sediment was used as inoculum in the previous studies that reported anaerobic degradation of estrogens. UASB sludge was used as inoculum in this study, and this might explain the lack of

degradation when compared to past studies. There have been several reports of the debromination of PBDEs by dehalogenating bacteria under anaerobic conditions (Gerecke *et al.*, 2005; He *et al.*, 2006; Xia, 2013), hence, our results may seem surprising. However, a study reported less than 20% anaerobic degradation of BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154 after 70 days with mixed culture inocula sourced from river sediment (Yen *et al.*, 2009). Another study also reported 30% debromination and degradation of BDE 209 within 238 days with digested sewage sludge under anaerobic conditions (Gerecke *et al.*, 2005). Both studies reported poor degradation of PBDEs after several months. This explains why degradation of PBDEs was not observed in this study, as the incubation period was comparatively short (15 days).

Predicting Effluent Quality and Associated Risks Using Obtained Degradation Rates

Using the equation given by (Levenspiel, 1999) described in **Supplementary Information S5**, the obtained biodegradation rates were used to predict effluent concentrations from previously sampled WWTPs (Komolafe *et al.*, 2021) and assess the likelihood of risk when these chemicals are discharged into water bodies accordingly. The assumptions for this assessment are detailed in Supporting Information (S5). This predicted effluent concentration was compared to the measured concentration of these chemicals reported previously (Komolafe *et al.*, 2021). The measured concentrations are combined aqueous and particulate phase concentrations.

The predicted concentrations were in agreement with the measured values (within the same order of magnitude) (**Table 3**, **Table 4**). The predicted effluent concentration for triclosan in both the activated sludge WWTP A (7,161 ng/L)

TABLE 3 | Predicted effluent concentration of the chemicals after activated sludge treatment (Brazil WWTP A).

Compound	Measured inf conc (ng/L)	Conc after sorption (ng/L)	Rate (h ⁻¹)	Predicted eff conc (ng/L)	Measured eff conc (ng/L)	EQS/PNECs (ng/L)
Triclosan	49,184	7,377.6	0.0072	7,161.0	1,486	100 ^a
Naphthalene	2,109.1	316.3	0.0340	276.8	200.8	130,000 ^c
Acenaphthylene	259.3	38.8	0.0180	36.1	55.8	-
Acenaphthene	347.2	52.0	0.0165	48.7	35.6	-
Fluorene	778.0	116.6	0.0134	110.4	75.2	-
Phenanthrene	2,119.1	317.8	0.0126	301.8	140.3	-
Anthracene	395.2	59.3	0.0082	57.3	67.0	100 ^c
Fluoranthene	1,135.2	170.3	0.0063	165.9	95.1	120 ^c
Pyrene	1,054.5	158.1	0.0038	155.6	100.5	-
Benz(a)anthracene	526.4	78.9	0.0050	77.3	90.5	-
Chrysene	459.2	68.8	0.0060	67.1	81.3	-
Benzo(b)fluoranthene	631.0	94.7	0.0053	92.7	57.7	17 ^c
Benzo(a)pyrene	393.3	59.0	0.0032	58.2	70.9	270 ^c
E1	78.2	n/a	0.1261	50.9	16.1	6 ⁺
E3	1,234.2	n/a	0.1426	771.6	64.3	60 ⁺

-the three HMW PAHs not included were not detected in the WWTP influent.

-Degradation rates were not obtained for PBDEs, hence they were excluded.

-E2 and EE2 were not detected in this WWTP, hence there were excluded.

-Conc represents concentration; inf and eff represents influent and final effluent respectively.

^aPNECs (UKTAG, 2013), ^cEQS (EU, 2013), + PNECs (Caldwell et al., 2012); - n/a means not applicable

TABLE 4 | Predicted effluent concentration of the chemicals after facultative pond treatment (WSP, Brazil WWTP C).

Compound	Measured inf conc (ng/L)	Conc after sorption (ng/L)	Rate (d ⁻¹)	Predicted eff conc (ng/L)	Measured eff conc (ng/L)	EQS standard (ng/L)
Triclosan	17,797.0	2,669.6	0.1752	592.7	924.0	100 ^a
Naphthalene	3,674.3	551.1	0.3046	77.7	124.7	130,000 ^c
Acenaphthylene	1,561.9	234.3	0.3337	30.5	47.6	-
Acenaphthene	770.8	115.6	0.3833	13.3	34.6	-
Fluorene	3,987.7	598.1	0.2641	95.2	68.2	-
Phenanthrene	7,658.8	1,148.8	0.2111	220.0	170.6	-
Anthracene	2073.3	311.0	0.0904	110.8	71.2	100 ^c
Fluoranthene	991.4	148.7	0.1024	48.8	127.5	120 ^c
Pyrene	4,073.5	611.0	0.0947	211.1	113.8	-
Benz(a)anthracene	723.3	108.5	0.0257	71.7	91.5	-
Chrysene	666.3	99.9	0.0253	66.4	82.8	-
Benzo(b)fluoranthene	541.4	81.2	0.0173	60.3	68.8	17 ^c
Benzo(a)pyrene	573.4	86.0	0.0385	48.6	66.3	270 ^c
E1	50.5	n/a	0.3888	5.8	0.1	6 ⁺
E3	625.1	n/a	0.1632	146.6	13.0	60 ⁺
EU WFD PBDEs	18.4	2.8	0.0655	1.2	12.8	140 ^c
BDE 209	251.2	37.7	0.0604	17.1	<10.8	4.8 ⁺

-the three HMW PAHs not included were not detected in the WWTP influent.

-E2 and EE2 were not detected in this WWTP, hence there were excluded.

-Conc represents concentration; inf and eff represents influent and final effluent respectively.

^aPNEC (UKTAG, 2013), ^cEQS (EU, 2013), +PNECs (Caldwell et al., 2012); - n/a means not applicable

and WSP (593 ng/L) was well above the predicted no-effect concentration (PNEC) value (100 ng/L). Also, the predicted concentration of naphthalene and benz(b)fluoranthene were below their EQS values in both WWTPs, while fluoranthene exceeded the EQS in the activated sludge WWTP, and anthracene exceeded in the WSP. For estrogens, the predicted concentration of E3 exceeded the PNEC values in both WWTPs, while that of E1 only exceeded in the activated sludge plant. The summed predicted concentration of EU Water Framework Directive (WFD) PBDEs (BDE 28, 47, 99, 100, 153, 154) was below the

EQS value in WWTP C (Table 4). Hence, overall, the effluent from these plants could pose a risk to aquatic organisms when discharged into receiving waters.

Some of these WWTPs would need unsuitable HRTs or river dilution to meet the EQS or PNEC values for some of the chemicals. For example, an HRT of over 10,000 h or river dilution by 62 times would be required for triclosan to meet PNEC concentrations in the activated sludge WWTP A while E1 will require 96 h or 9 times dilution (Supplementary Table S8). Neither long HRTs nor river dilution are suitable mitigation

strategies, however, which would suggest that tertiary treatments are required for effluents of these WWTPs.

CONCLUSION

Degradation of all the chemicals was only observed under aerobic conditions with a faster reaction kinetic with activated sludge inocula over the facultative inocula from the WSP. The degradation kinetics of the chemicals was in the following order: estrogens > LMW PAHs > triclosan > PBDEs > MMW PAHs > HMW PAHs. The results suggested that biodegradation is a major removal mechanism during biological treatment in the activated sludge and WSP WWTPs surveyed. However, volatilization was also observed to contribute to the removal of some of the chemicals within these two treatment systems. Furthermore, the susceptibility of chemicals to photodegradation differed between and within classes of chemicals-PAHs and estrogens degraded faster under light conditions (white fluorescent light) while triclosan and PBDEs degraded faster in the dark. Degradation of all test chemicals was not observed under anaerobic conditions with the UASB sludge inocula, suggesting that their removal - if any - observed during UASB treatment was most likely due to sorption to sludge and not to biodegradation. Sorption is an important removal pathway of these chemicals in treatment systems that should be investigated thoroughly in a future study to understand the overall fate of the chemicals.

The first order degradation rates obtained in this study for different classes of chemicals including triclosan, PAHs, estrogens and PBDEs was successfully used to predict effluent concentrations and estimate required HRTs for removal of these chemicals to concentrations below current EQS/PNEC values. Importantly, there was a good match between the predicted and measured effluent concentration for all the chemicals investigated. These concentrations were above the EQS/PNEC values for some of the chemicals in all classes, and the required HRT for adequate removal is mostly impractical. However, river dilution might ensure compliance for some of the chemicals under certain circumstances, although this is not really a desirable or suitable mitigation strategy. Furthermore, the synergistic effect of the cocktail of different classes of compounds on aquatic organisms remains unknown. This work suggests that improvements in these technologies and/or other tertiary treatment technologies are required to ensure such systems can achieve environmentally safe levels of some micropollutants in receiving water courses.

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

OK: Conceptualization, Methodology, Data acquisition, Formal analysis, Investigation, Writing- original draft. WM: Conceptualization, Supervision, Analytical Methodology, Writing-review and editing. KA: Investigation, Writing-review and editing. LV: Resources, Writing-review and editing. CM: Supervision, Writing-review and editing. RD: Funding acquisition, Conceptualization, Supervision, Writing-review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2021.700245/full#supplementary-material>

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Conflict of Interest: Author OK was employed by GFL Environmental Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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